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In vitro antifungal activity, phytochemical screening and thin layer chromatography profiling of *Impatiens tinctoria* A. Rich root extracts

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Abstract
Background: The emergence of microorganisms' new resistance mechanism, the emerging and re-emerging of new infectious disease and side effects of antimicrobials threatening our ability to treat infectious diseases. Therefore, researches for the innovation of new drugs are needed through identification of the best candidate like plants which are used in traditional medicine. In Ethiopia, women dye their palms and nails by a root paste of the study plant (*Impatiens tinctoria* A. Rich) as a beauty treatment, to toughen the skin and to control fungal infections.
Objectives: To investigate the antifungal activities, phytochemical screening and Thin Layer Chromatography profiling of aqueous, ethanol and ethyl acetate root extracts of *Impatiens tinctoria* A. Rich.
Methods: The roots of *Impatiens tinctoria* A. Rich were collected from Gurage Zone around Butajira town and processed and extracted using solvents of ethyl acetate, ethanol and water. Agar well diffusion and agar dilution methods were used for screening the antifungal activity of the extracts and for determination of minimal inhibitory concentration, respectively. The minimum fungicidal concentration of the extracts was determined and the plant extracts were subjected to phytochemical screening.
Result: From the tested fungi *T. rubrum* and *T. mentagrophytes* were more susceptible. The study screened the presence of at least 7 phytochemicals (alkaloids, flavonoids, anthraquinens, terpenoids, glycosides, quinones and saponins) and 6 compounds in the roots of the study plant. Ethyl acetate extract was more potent and broader spectrum of antifungal activities than ethanol and aqueous extracts.
Conclusion: The roots of the study plant showed notable antifungal potency. However, further research should be undertaken to elucidate the involved phytochemicals and compounds present in the extracts and mechanism of action which clear out the road for developing new antifungal drugs.

Keywords: Antifungal, phytochemicals, thin layer chromatography, root extracts, *Impatiens tinctoria* A. Rich, Ethiopia

1. Introduction
Antimicrobial resistance becomes a global concern because new resistance mechanisms are emerging and spreading globally, threatening our ability to treat common infectious diseases [1]. In the other way there is a major challenge to combat new emerged infections since there is no specific treatment against them [2]. In addition to these problems, drugs are sometimes associated with adverse effects on host which include hypersensitivity, depletion of beneficial gut and mucosal microorganisms, immune suppression and allergic reactions [3]. Moreover, in the last few decades drug development is becoming more and more inefficient mainly due to large development costs and low clinical trial drugs success rate (below 12%) [4]. Due to these problems the burden of infectious diseases like fungal infections is growing globally [5].
Though only 15% of the angiosperms (flowering plants) have been chemically investigated for their medical potential, various important drugs were developed from plants and their secondary metabolite constituents [6-8]. The leading effect of traditional healing practices on medicinal plants contribute the discovery of about 25% of modern medicines (like emetine, quinine and berberine), that is, descended from plants primarily used as traditional remedies [8].

Medicinal plants are now more focused than ever due to the isolation of antimicrobial chemicals from them such as alkaloids, terpenoids, flavonoids, tannins and phenolic compounds [9-13]. Therefore, using plants for innovation of new drugs by studying the potential antimicrobial activity of plant derived substances which are used in traditional medicine in different countries is a good alternative solution to treat infectious diseases [8].

The study plant *Impatiens tinctoria* A. Rich belongs to *Impatiens* genera and that of Balsaminaceae family is one of the species of over 1000 species belonging to this family [14]. It is known by English vernacular name, Balsamine or by the vernacular name in Amharic, "Insosila". It is an upright perennial herb that grows to approximately 2 metres tall. It develops a large tuberous rootstock that lies at or just below the soil surface (figure 1) [15]. It is native to East Africa and found many parts of Ethiopia [16]. Flora biodiversity assessment studies recorded as *I. tinctoria* A. Rich is one of the most abundant herb species in Bonga Forest, Oromia Region of Ethiopia and in Mahoney / Maichew, Southern Tigray Region of Ethiopia has been found out as the dominant cash herb [17, 18].

Fig. 1: The *I.tinctoria* A. Rich A) parts above the ground B) Roots

In Ethiopia women chop or mash the inside of the tubers (roots) of *I. tinctoria* A. Rich in to a paste to dye the palms and nails of the hands and feet a dark reddish color (Figure **2**). It is considered a beauty treatment similar to that of henna. It also helps to control fungal infections like ringworm that cause athletes foot (tinea pedis) and to toughen the skin. [15,19]. Thus, using this information and other ethnobotanical studies [20-22] that support the medicinal value of the plant as a base line the root extracts of the study plant was evaluated for antifungal activities. The preliminary phytochemical screening could be used as a starting point to quantify the identified phytochemical constituents and for further isolation of bio-active compounds. So, the study provides base line information for the development of alternative new drugs.

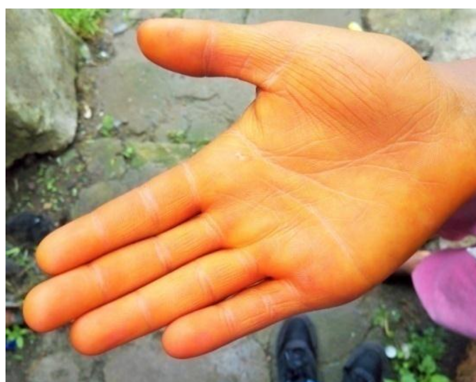

Fig. 2: Dyed palms by a paste of *I.tinctoria* A. Rich roots

2. Materials and methods

2.1 Plant material collection

The roots of *I. tinctoria* A. Rich were collected around Butajira town after obtained permission from Butajira town administration. Butajira is located in the Gurage Zone of the Southern Nations, Nationalities and Peoples' Region, Southern central Ethiopia, 130 km from Addis Ababa. This town has a latitude and longitude of 8°07′N 38°22′E and an elevation of 2131 meters above sea level. The plant material was identified by a botanist, and a voucher specimen deposited in the herbarium of Traditional and Modern Medicine Research Directorate (TMMRD).

2.2 Herbal material preparation

Fresh *I. tinctoria* A. Rich roots were washed with clean water and rinsed with distilled water, cut into pieces and dried at room temperature in shade and lastly milled to powder by using milling machine. Then, the powder was weighed using electronic weighting balance and packed in polyethylene bags to avoid entrance of air and any other contaminant and stored in closed container with proper labeling for further extraction processes.

2.3 Extraction of the collected roots

Different studies on medicinal plants showed as extraction solvents used for extraction has a great influence on the antimicrobial activity of the plant materials [23-25]. Therefore, the powder part of the study plant was extracted using three different solvents. 100 grams of the powdery sample was weighed into each of the three cleaned and dried 2000 ml reagent bottles and 1000 ml of each solvent (ethyl acetate, ethanol and water) was separately added to each of the bottle and shaken (100 revolution per minute) on a shaking orbit machine (VWR DS-500; The Lab World Group, Boston, MA, USA) for 24 hours at room temperature. The mixture was filtered through whatman no.1 filter paper. After filtration the ruminant was repeated for additional two cycles with fresh solvent. The ethyl acetate and ethanol filtrate was concentrated under reduced pressure at 40ºC by a rotary evaporator (R-200 Buchi, Switzerland). These concentrated filtrates were transferred to beakers and dried by keeping beakers in water bath by setting at 40 ° C to avoid the

remaining organic solvents while water filtrate dried by lyophilizer (freeze dryer). Finally, extracts were kept at 2-8°C [26].

2.4 Antifungal activity of the extracts

2.4.1 Microorganisms

Antifungal activity of *I. tinctoria* A. Rich root extracts were evaluated on clinical isolated yeast (*C. albicans) and* on four drug sensitive standard strains of American Type Culture Collection (ATCC) molds that were obtained from microbiology laboratory of TMMRD and National Clinical Bacteriology and Mycology Reference Laboratory of EPHI, Ethiopia. The tested mold strains were *T. rubrum* (ATCC 28188), *T. mentagrophytes* (ATCC 18748), *A. niger* (ATCC 10535) and *A. flavus* (ATCC 13697).

2.4.2 Inoculum preparation

Inoculums preparation was according to recommended by Clinical and Laboratory Standards Institute (CLSI). All strains were grown in Petridishes containing sabroud dextrose agar medium as refreshment of each strain for actual test by incubating at 25°C for 7 days. Standardization was by taking 3-5 inoculums from a fresh, pure culture of the test organism and making a suspension with sabroud dextrose broth. The absorbance of the prepared suspension was read by uv-visible spectrophotometer (Thermo Scientific Evolution 60s CAT 840210100) with a 1cm light path till obtained an absorbance reading of 0.08 to 0.1 at 625nm which is proportional to $1x10^7$ spores/ml fungi. Then, these suspension diluted with appropriate broth in 1:10 to get $1x10^6$ spore/ml fungi, respectively that used to evaluate the antifungal activity of the extracts parallel to positive and negative controls [27, 28].

2.4.3 Screening antimicrobial activity of the extracts

Agar well diffusion technique was used for screening of antimicrobial activity of the plant extracts. A sterile cotton swab was dipped into the adjusted suspension. The swab rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This removes excess inoculums from the swab. The dried surface of a plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculums. As a final step, the rim of the agar swabbed [27].

The streaked plate punched aseptically with a sterile cork borer that has a diameter of 8mm to form a hole for placing extracts, positive and negative controls. A 100µl volume of the antimicrobial agent or extract solution at desired concentration (100mg/ml, 200mg/ml and 400mg/ml) was introduced into the well. Standard drugs as positive control and diluents as negative control were run simultaneously. Then, the plates incubated at 25°C up to 7 days. The antifungal agent diffuses in the agar medium and inhibits the growth of the fungal strains tested. The presence of inhibition zones were measured by ruler recorded and considered as indication for antifungal activity [28].

2.4.4 Determination of Minimum Inhibitory Concentration

Standard agar dilution method was employed to determine Minimum Inhibitory Concentration (MIC) value of the plant extracts against all screened 5 test organisms. Different concentrations of extracts starting from a highest concentration of 64 mg/ml to 0.0625 mg/ml were prepared by two-fold serial dilution. Then, 2ml of the prepared concentrations were added in to test tubes containing 18 ml of molten agar medium that cooled to 55ºC and mixed thoroughly followed by dispensing to the petridish. After dispensing the plates were allowed to solidify at room temperatures. The next procedure was putting inoculums by taking 2µl standardized suspension using micropipette. The inoculums suspension preparation and incubation time was similar to the screening agar well method. The MIC was considered as the lowest concentration which inhibits the growth of the respective fungi under suitable incubation conditions expressed in mg/ml [29].

2.4.5 Determination of Minimum Fungicidal Concentration

Streaks were taken from MIC plates exhibiting invisible growth and sub cultured onto appropriate extract and standard drug free agar plate medium. The plates incubated at 25°C up to 7 days. Then after examined for fungal growth in corresponding to plant extract concentrations in which Minimum Fungicidal Concentration (MFC) was taken as the highest diluted extract that showed absence of any growth [30].

Generally, during the above all antimicrobial assays positive controls, negative controls, sterility controls and growth controls were run parallel to the experimental tests. Discs of amphotericin B (32µg) were used as positive control in well diffusion assays whereas by diluting the powdered form of these standard drugs was used for MIC value determination. Since ethanol and ethyl acetate extracts were dissolved by 5% tween 80 and aqueous extract was dissolved by distilled water, 5% tween 80 for ethanol and ethyl acetate extracts and distilled water for aqueous extract were used as negative control. All assays were performed in triplicate.

2.5 Phytochemical screening

The qualitative phytochemical investigation of each extract of the herbal material was performed to evaluate the presence of different classes of secondary metabolites such as alkaloids, flavonoids, phenols, saponins, tannins and terpenoids. The qualitative results were expressed as positive for the presence and negative for the absence of phytochemical/s. It was carried out using standard tests as described below.

Test for alkaloids (Wagner's Test): Extracts were dissolved individually in dilute HCl and filtered. Then**,** filtrates of each extract were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids [10].

Test for flavonoids (H_2SO_4 test): A fraction of the extracts was treated with concentrated H_2SO_4 and observed for the formation of orange color [10].

Test for phenols: About 0.5 g of plant extract was added to 1 ml of 10% $FeCl_3$ solution. A deep bluish green coloration was an indication for the presence of phenol [31].

Test for tannins: 0.5 g of plant extract was mixed with 2mL of water and heated on water bath. The mixture was filtered and 1mL of 10% $FeCl_3$ solution was added to the filtrate. A blue-black solution indicates the presence of tannin [32].

Test for free anthraquinones (Borntrager's Test): About 0.2 g of each extract was shaken with 10 ml of benzene and then filtered. Five ml of the 10% ammonia solution was then added to the filtrate and shaken. Appearance of a pink, red or violet color in the ammonia (lower) phase was taken as the presence of free anthraquinones [32].

Test for coumarins: To the 2ml of extract 10% NaOH was added and shaken well for 5minutes shows the yellow color that indicates the presence of coumarins [33].

Tests for quinones: To the 2ml of extract a concentrated H2SO4 added and shaken well for 5 minutes shows the red color that indicates the presence of quinones [33].

Test for Saponins: To 1ml extract 20ml distilled water was added and shaken well in measuring cylinder for 15min. Formation of 1cm layer of foam indicates the presence of saponins [34].

Test for terpenoids (Salkowski test): The extract was mixed with 2ml of chloroform and concentrated H_2SO4 (3ml) is carefully added to form a layer. A reddish brown coloration of the interface shows positive result of the presence of terpenoids [34].

Test for glycosides: In 5ml extract, 2ml glacial acetic acid, one drop of 5% FeCl3 and conc. H_2SO4 were added. Appearance of brown ring indicates presence of glycosides [34].

2.6 Thin Layer Chromatography profiling

For Thin Layer Chromatography (TLC) analysis TLC plates with Silica gel matrix on it with a layer thickness (200 µm), particle size (2-25 µm) and pore size (60 Å medium pore diameter) was used by cutting the 20×20cm plate sheet to 1×10cm strip. Plate markings were made with soft pencil. A half gram of the crude extracts of ethyl acetate, ethanol and water were dissolved in their respective solvent that is with 2 ml of ethyl acetate, ethanol and water, respectively. Capillary tube was used to spot a 2 drop of sample solution on the silica gel TLC plate at 1cm from the edged of the plate and the drop was allowed to dry [35].

The solvent systems used were hexane: ethyl acetate in a ratio of 7:5 for ethyl acetate and ethanol extract, dichloromethane: ethyl acetate in the ratio of 7:2 for water extract by adding a few drop of acetic acid to each prepared solvents. The chambers (for each solvent system) were allowed to saturate for at least 20 minutes before the TLC plates were placed. After the solvent front reached 3/4th of the TLC height, the plates were removed from the chamber and allowed to dry. The plates were then observed in a UV fluorescent reader at 254 nm wavelengths. This TLC was done to provide a finger print of the crude extract by providing a rough number of components and nature in the extract indicated by the bands on the plate [35].

2.7 Data Quality Assurance

To ensure the quality of the data all the laboratory works were performed according to the SOPs and internationally accepted principles for testing of medicinal plants by the principal investigator with the help of experienced professionals. Each equipments, supplies, reagents and procedures were adequately controlled. The assessment of the antimicrobial activity, the TLC profiling and phytochemical screening of the extracts was performed at least in triplicate with controls for insuring quality and reproducibility results. There was a continuous monitoring and supervision of the data extraction by advisors. The extracted data was examined for completeness and checked for consistency before entering it in to the excel spreadsheet.

2.8 Data analysis and interpretation

Data was entered into excel spreadsheet and analyzed using Minitab 16 software. The statistical differences of the antifungal activity of crude extract on each fungi was carried out by employing one way analysis of variance (Anova) followed by Tukey's multiple comparison tests. The experimental data was expressed as mean ± Standard Deviation (SD). The result was considered statistically significant at 95% confidence level and $P<0.05$.

3. Results

3.1 Antifungal activity screening by well method

On this method the study plant root extracts showed different inhibition zone against *C. albicans, T. rubrum, T. mentagrophytes, A. niger and A. flavus*. The dermatophytes, *T. rubrum* and *T. mentagrophytes,* were more susceptible compared to the other fungal species at all tested extract types and concentrations. Ethyl acetate extract showed a higher inhibition of the growth of tested fungi when compared to aqueous and ethanol extracts ($P<0.05$). The inhibition activity of each extracts was dose dependent. There was no inhibition of *C. albicans, A. niger* and *A. flavus* against the aqueous crude extract at 100mg/ml concentration. Among the extracts used aqueous extract has showed significantly lower inhibition for all tested fungi except *A. niger* ($P<0.05$). Generally, the antifungal activities of the ethyl acetate extract(at all tested concentrations) in terms of its zone of inhibition were better or in line with the positive amphotericine B (32 µg /ml) against each fungal strain (table 1).

Table 1: Inhibition zone diameter measurement (mm) of crude extracts of *I. tinctoria* A. Rich roots against fungi

Extracts Type	Conc. (mg/ml)	Inhibition Zone Diameter (mm), including well diameter (8 mm)				
		C. albicans	*T. rubrum*	*T. menta*	*A. niger*	*A. flavus*
Aqueous	100	8.0 ± 0.0^{f}	14.0 ± 1.0^{d}	12.7 ± 0.6^{g}	8.0 ± 0.0^{f}	8.0 ± 0.0^{h}
	200	$8.7\pm0.6e^{f}$	26.0 ± 1.0^{c}	16.7 ± 1.5^{f}	9.3 ± 0.6^{ef}	9.0 ± 1.0^{gh}
	400	$9.7\pm0.6de^{f}$	29.0 ± 1.0^{b}	26.7 ± 1.5^{cd}	11.3 ± 1.2^{de}	10.7 ± 0.6^{ef}
Ethanol	100	11.3 ± 0.6^{de}	29.0 ± 1.0^{b}	25.0 ± 1.0^{d}	9.7 ± 0.6^{ef}	10.0 ± 0.0^{fg}
	200	12.3 ± 0.6^{d}	31.3 ± 0.6^{b}	28.7 ± 1.2^{bc}	11.0 ± 1.0^{de}	11.3 ± 0.6^{ef}
	400	15.7 ± 0.6^{c}	35.7 ± 0.6^{a}	30.3 ± 0.6^{d}	13.0 ± 1.0^{d}	12.0 ± 0.0^{e}
Ethyl acetate	100	22.7 ± 1.5^{b}	28.7 ± 0.6^{bc}	29.0 ± 1.0^{bc}	22.3 ± 0.6^{b}	20.3 ± 0.6^{c}
	200	24.7 ± 1.2^{ab}	31.3 ± 1.5^{b}	31.3 ± 1.2^{ab}	24.3 ± 0.6^{b}	26.7 ± 0.6^{b}
	400	27.3 ± 2.1^{a}	36.7 ± 1.5^{a}	33.7 ± 1.2^{a}	27.3 ± 0.6^{a}	28.7 ± 0.6^{a}
Distilled water		8.0 ± 0.0^{f}	8.0 ± 0.0^{e}	8.0 ± 0.0^{h}	8.0 ± 0.0^{f}	8.0 ± 0.0^{h}
5% tween 80		8.0 ± 0.0^{f}	8.0 ± 0.0^{e}	8.0 ± 0.0^{h}	8.0 ± 0.0^{f}	8.0 ± 0.0^{h}
Amphotericin B	32 µg	25.3 ± 0.6^{ab}	13.3 ± 0.6^{d}	20.3 ± 0.6^{e}	20.0 ± 1.0^{c}	15.7 ± 0.6^{d}

Key notes: Values are expressed as Mean ± SD (n=3), 8.0±0.0 = no inhibition (well diameter), "Means" that do not share a superscript letter are significantly different (only column wise) at $P<0.05$. *Conc.-concentration, T. menta. - T.mentagrophytes*

3.2 Minimum Inhibitory Concentration and Minimum Fungicidal Concentration of the extracts
Among the tested fungi, *T. rubrum* was the most susceptible with MIC value of 0.7±0.3 mg/ml, 1.0±0.0 mg/ml and 1.0±0.0 mg/ml by ethyl acetate, ethanol and aqueous extracts, respectively, followed by *T.mentagrophytes* which has showed MIC value of 1±0.0 mg/ml, 4±0.0 mg/ml and 8±0.0 mg/ml, respectively. *C. albicans, A. niger* and *A. flavus* were more resistant fungal species to the extracts with MIC value of greater than 64 mg/ml for both ethanol and aqueous extracts. However, ethyl acetate extract the MIC value of 16±0.0 mg/ml and 32±0.0 mg/ml were recorded for *C.albicans* and *A. flavus*, respectively (table 2). The MFC value was in agreement with its MIC, that is, the lower MIC value is the lower MFC value in most of the tested microorganisms.

Table 2: MIC and MFC value of tested fungi

Microorganisms MIC and MFC		Water extract	Ethanol extract	Ethyl acetate extract	Positive control
C.albicans	MIC	>64±0.0	>64±0.0	32±0.0	32±0.0
	MFC	>64±0.0	>64±0.0	64±0.0	64±0.0
T.rubrum	MIC	1±0.0	1±0.0	0.7±0.3	2±0.0
	MFC	2.7±1.2	2±0.0	1±0.0	4±0.0
T.mentagrophytes	MIC	8±0.0	4±0.0	1±0.0	2.7±1.2
	MFC	16±0.0	8±0.0	2±0.0	4±0.0
A.niger	MIC	>64±0.0	>64±0.0	16±0.0	6.7±2.3
	MFC	>64±0.0	>64±0.0	16±0.0	8±0.0
A.flavus	MIC	>64±0.0	>64±0.0	32±0.0	16±0.0
	MFC	>64±0.0	>64±0.0	32±0.0	32±0.0

Key notes: Amphotericin B was the positive control drug. The MIC and MFC values were expressed in mg/ml for extracts and in µg/ml for positive control

3.3 Phytochemical constituents of the crude extracts
According to the qualitative phytochemical screening study, around 7 secondary phytochemicals were detected at least in one of the three extracts (table 3). The ethyl acetate, ethanol and aqueous extracts of the roots of *I.tinctoria* A.Rich were found to be positive for 6, 5 and 4 tested secondary metabolites, respectively. Glycoicides, saponins and terpenoids were positive for all extracts whereas coumerines, phenols and tannins were negative for all extracts.

Table 3: Secondary metabolites detected with in aqueous, ethanol and ethyl acetate crude extracts of the root *I. tinctoria* A.Rich

Phytochemical constituent	Water extract	Ethanol extract	Ethyl acetate extract
Alkaloids	-	+	+
Free anthraquinens	+	-	-
Coumerines	-	-	-
Flavoinoids	-	-	+
Glycoicids	+	+	+
Phenols	-	-	-
Quinines	-	+	+
Saponin	+	+	+
Tannins	-	-	-
Terpenoids	+	+	+

Key notes: (+) positive, (-) negative

3.4 TLC profiling
TLC analysis of each extract was performed using various combinations of solvent systems till the analysis revealed a better separated constituents. The solvent system containing a mixture of hexane: ethyl acetate (7:5) produced a better separation for ethanol and ethyl acetate extracts. Though various solvent combinations with different proportions tested, the aqueous extract didn't give well separated constituents of compounds. Of the analyzed mixture dichloromethane: ethyl acetate (7:2) produced a little bit better separated components. The migrated constituents, formed by the solvent system, observed under UV-light at a wavelength of 254nm, revealed the presence of 6, 4 and 1 compounds with in ethanol, ethyl acetate and aqueous extracts, respectively (Figure 3).

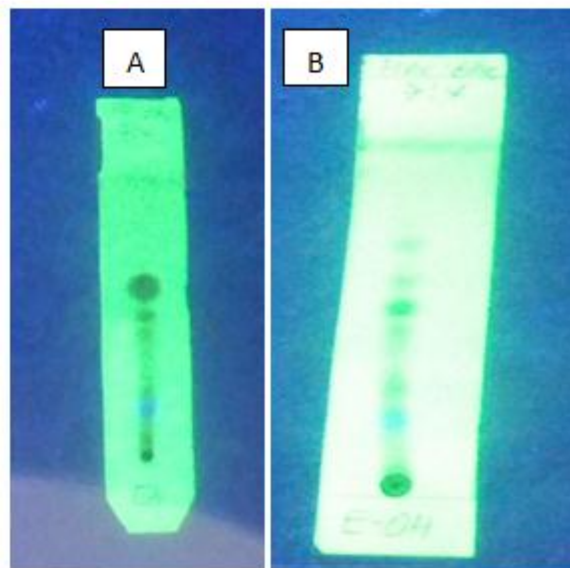

Fig 3: TLC fingerprint of A) Ethyl acetate extract and B) Ethanol extract

4. Discussion
The well method assay showed various inhibition diameters of the tested microorganisms by all of the extracts with the exception of 100 mg/ml aqueous extract that did not show any inhibition for *C. albicans, A. niger* and *A. flavus.* Most of the tested fungi inhibition by extracts showed statistically significant difference ($p<0.05$) between concentrations of 100mg/ml and 400mg/ml respected extracts in which higher concentration records longer inhibition zone than lower concentration, i.e., antimicrobial activity increased with the increase in the concentration of the extracts. This indicates as the extracts could contain antifungal constituents, whose concentration increased with dose and possibly increase the activity till it reaches its maximal efficacy that might be ≥400mg/ml.

At 400mg/ml concentration *C. albicans, A. niger* and *A. flavus* inhibited with a diameter of 9.7±0.6mm, 15.7±0.6mm and 27.3±2.1mm by aqueous, ethanol and ethyl acetate extracts, respectively. The 100mg/ml of aqueous extract did not show any antifungal activity against these fungi. These results were too low compared to *T.rubrum* and *T. mentagrpophytes* that showed a lowest inhibition zone of 14.0±1.0 and 12.7±0.6mm by 100mg/ml aqueous extracts and a highest inhibition zone of 36.7±1.5mm and 33.7±1.2mm by 400mg/ml of ethyl acetate extracts, respectively. MFC and MIC values also support these results and showed the more susceptibility of *T.rubrum* and *T. mentagrpophytes* compared to the above three tested fungi's. Therefore, *t*he application of the paste of the study plant roots by Ethiopian females as a treatment of tinea [15] which is caused by primarily by *T.rubrum* followed by *T. mentagrophytes* and other dermatophytes [36, 37] supported by this findings as the extracts of *I.tinctoria* A.Rich endowed good antifungal effect against

these most prevalent Trichophytons (*T.rubrum* and *T.mentagrophytes)* [38] than the other tested fungi.
Of the screened 10 phytochemicals seven were positive in which some were positive for the three extracts or two extracts and others for one extract of *I.tinctoria* A.Rich roots. Therefore, the phytochemical composition of the three extracts differ by type and those similar type phytochemicals that found in different extracts might also differ by concentration. Different studies support the extraction solvents used for extraction has a great influence on the type and concentration of phytochemicals screened [39, 40]. The solvents used in the extraction of the plant materials might not only contribute for the difference in kind of these secondary metabolites among the three extracts but might also contribute for the difference in concentration.
The study revealed the presence of terpenoids, saponins and glycoicides with in the three extracts of *I. tinctoria* A. Rich roots. Previously, numerous phytochemicals such as alkaloids, anthraquinens, flavonoids, terpenoids, glycosides, quinones and saponins have also revealed from many medicinal plants that had health benefits such as antimicrobial effect through different mechanisms [12, 32, 41]. For instance, a study on the isolate of terpenoides from the medicinal plant *Trichodesma amplexicaule* Roth found the presence of antifungal activity of this phytochemical [42]. Therefore, the isolated phytochemicals from the root extracts of the study plant might help for the potency of antifungal activity.
Alkaloid was the other phytochemical screened from ethyl acetate and ethanol extracts but not in aqueous extract. The presence of this phytochemical in the ethyl acetate and ethanol extract could contribute for their better antimicrobial activities than the aqueous extract. This idea supported by a study in Slovakia on antibacterial and antifungal effect of Mahonia aquifolium crude extract and its major isolated alkaloids berberine and jatrorrhizine on 20 bacterial and fungal tested strains revealed that the isolated alkaloids berbeine showed more susceptibility on most the microorganisms [43]. In addition to the above phytochemicals only ethyl acetate extract was positive for flavoinodes that might have a great contribution for its antifungal activity as different studies support the antimicrobial effect of this phytochemical [33, 44].
The study detected greater number of phytochemicals in ethyl acetate extract than ethanol and aqueous extracts. Of the three extracts the ethyl acetate extract has stronger and broader spectrum of antimicrobial activities as observed from mean inhibition diameter measurements and the consistent MIC and MFC values. This might be due to the synergistic effect of the secondary metabolites as many studies assure synergistic effect of secondary metabolites increase the antimicrobial potency of plant extracts [45-47]. Additionally, the bioactive phytochemicals that only found in ethyl acetate extract might have relatively more antimicrobial activity than others. On the other way not only by type but also the concentration of the phytochemicals present in the ethyl acetate extract might be greater than ethanol and aqueous extracts that could increase the antimicrobial activity of ethyl acetate extract than ethanol and aqueous extracts.

5. Conclusion
According to this study, the plant extracts showed varying antifungal activities in which *T.rubrum* and *T.mentagrophytes* were more susceptible at all corresponding extract types and concentrations compared to other tested fungi. Therefore, this antifungal profile of the study plant on this study support applying of *I.tinctoria* A.Rich roots paste topically for control of certain fungal infections though further studies are needed. The antifungal activity of the study plant showed notable extraction solvent dependent manner in which ethyl acetate extract showed more antifungal activity in most of the tested fungi compared to the aqueous and ethanol extracts. This might be due to the type and concentration of phytochemicals present in each extract.
Ethics approval and consent to participate
Not applicable
Consent for publication
Not applicable

Competing interests
No competing interests

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Availability of data and materials
All raw data were generated at Ethiopian Public Health Institute. The corresponding author has all fabricated data that support the findings of this study and majority of them are presented in the main manuscript.

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